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Effect of peritubular capillary perfusion rate on proximal sodium reabsorption

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Effect of peritubular capillary perfusion rate on proximal sodium reabsorption. The effect of changes in peritubular capillary flow rate on fluid reabsorption by the renal proximal tubule was studied in rats. Absolute and fractional water reabsorption was measured by the recollection technique in end proximal convolutions while the adjacent peritubular capillaries were being microperfused at different rates. Pooled rat plasma with a protein concentration of either 5.3 or 11.6 g/100 ml was used to perfuse the capillary network branching from the efferent arteriole at the center of a vascular "star". Fractional and absolute reabsorption fell consistently when the capillary perfusion rate was raised. This occurred with both the low and high protein solutions. When the capillaries were perfused first at a high rate and then at a lower rate, absolute and fractional reabsorption rose during the second tubular collection, suggesting that the results were not due to an artifact of the recollection method or non-specific effects of capillary perfusion. Although part of the inhibition observed with the low protein solution might have been due to dilution of the normal peritubular capillary proteins (a colloid oncotic effect), the magnitude of inhibition seemed too great to be accounted for by this alone. Furthermore, since inhibition was also found when the hyperoncotic plasma was used to perfuse the capillaries at a high rate, a factor other than colloid oncotic pressure changes seemed evident. The observations are consistent with the view that changes in capillary hydrostatic pressure produced by the different perfusion rates played a role in the changes in sodium and water reabsorption under these experimental conditions.

Effet du débit de perfusion capillaire péritubulaire sur la réabsorption proximale du sodium. L'effet des variations du débit capillaire péritubulaire sur la réabsorption du sodium par le tube proximal rénal a été étudié chez des rats. La réabsorption d'eau absolue et fractionnelle a été mesurée à la fin des tubes proximaux accessibles à différents débits de perfusion des capillaires péritubulaires en utilisant la technique des recollections. Du plasma de rat provenant de pools contenant soit 5,3 soit 11,6 g/100 ml de protéines a été utilisé pour perfuser le réseau capillaire issu d'une artérioleéfférente au centre d'une étoile vasculaire. Les réabsorptions fractionelle et absolue ont diminué nettement quand le débit de perfusion capillaire a été augmenté. Cela a été observé avec les deux concentrations de protéines. Quand les capillaires ont été perfusés d'abord à un débit élevé puis à un débit plus faible, les réabsorptions absolue et fractionnelle ont augmenté au cours de la deuxième collection, ce qui suggère que les résultats ne sont pas dus à un artifice de la technique de recollection ou à un effet non spécifique de la perfusion capillaire. Une partie de l'inhibition observée avec la solution à faible concentration de protéines peut aboir été liée à la dilution des protéines propres du capillaire péritubulaire (effet colloïdo-oncotique). Cependant l'importance de l'inhibition semble trop grande pour que ce processus en tende compte exclusivement. De plus, le fait que l'inhibition ait été obtenue aussi lors de la perfusion du capillaire, à débit élevé, avec du plasma hyperoncotique rend évident l'existence d'un facteur autre que les modifications de pression colloïdo-oncotique. Ces constatations sont compatibles avec l'idée que les modifications de la pression hydrostatique capillaire produites par les différents débits de perfusion jouent un rôle dans les modifications de la réabsorption du sodium et de l'eau dans les conditions expérimentales utilisées.

In a number of previous studies [1–5] it was found that the reabsorptive rate for sodium and water by the proximal convoluted tubule tends to parallel the glomerular filtration rate (GFR) under a variety of experimental conditions in which acute changes in GFR were induced. This relationship in which fractional reabsorption remains relatively constant as GFR changes has been termed "glomerulotubular balance". The mechanisms responsible for the phenomenon are as yet incompletely understood. Recent observations suggest, however, that factors other than the volume of fluid delivered into the tubular lumen can be of critical importance in determining fluid reabsorption [6–12]. It has been postulated that the absolute rate of sodium and water reabsorption by the proximal tubule is regulated at least in part by physical forces which determine fluid exchange across the peritubular capillaries, and that these forces may or may not be altered by experimental maneuvers which change GFR [6, 8, 9, 12, 13].

In the present experiments, we have studied the effect of acute changes in peritubular capillary flow rate on fluid reabsorption by the proximal convoluted tubule of the rat kidney, using micropuncture techniques. Pooled rat plasma with a protein concentration of 5.3 g/100 ml, or rat plasma

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concentrated to a protein concentration of 11.6 g/100 ml was used to perfuse the peritubular capillaries and free-flow recollections of tubular fluid were made from the end proximal convolutions supplied by the perfused capillary network. We found that both fractional and absolute sodium reabsorption by the proximal tubule were inversely related to the perfusion rate. The changes in reabsorptive rate induced by capillary perfusion were independent of the glomerular filtration rate of the nephron. Although the precise mechanism was not determined, the findings are consistent with the view that changes in capillary hydrostatic pressure mediated the changes in sodium reabsorption.

Methods

Micropuncture experiments were carried out on white male rats weighing 225 to 325 g. The animals were fed a regular rat pellet diet and allowed free access to water. Food but not water was withheld the night before an experiment. The methods of anesthesia and surgical preparation of the animals have been described previously [14]. Blood pressure was monitored continuously via a PE 50 catheter in a carotid artery leading to a Statham strain gauge (P 23Dc) and Grass polygraph (Model 5D). Body temperature was maintained at 37 to 38° C by a heated animal table (rectal temperature monitored continuously by a telethermometer, Yellow Springs Instrument Company, Inc.). Intravenous infusion of Ringer's lactate was administered continuously at 0.05 ml/min via a jugular vein. An additional volume of fluid equal to one percent of body weight was given at the end of the surgical preparation in order to replace fluid loss from the surgically exposed tissues. The left ureter was catheterized (experimental kidney) with PE 50 tubing for collection of timed urine specimens and measurement of inulin clearance. Tritiated methoxy inulin (New England Nuclear Corp., Boston, Mass.) was given in a priming dose of 25 μ Ci and a sustaining dose of 0.4 μ Ci/min was added to the i.v. infusion. In a preliminary study, shown in Table 1, clearances of ^{3}H-methoxy inulin and ^{14}C-carboxyl inulin were measured simultaneously in the same animal and were found to yield identical results.

The methods used to perfuse surface capillaries and to collect tubular fluid simultaneously were essentially the same as those described by Spitzer and Windhager [12] with certain modifications. In order to perfuse the capillaries in the same direction as the normal blood flow, we perfused the efferent arteriole at the center of vascular "stars" since it has been demonstrated that blood flows upward from beneath the surface of the kidney in these arterioles and outward into the branches of the star [15]. The end proximal convolutions and vascular "stars" were identified by means of intravenous injection of a small bolus of 10% Lissamine green. Pooled rat plasma (albumin 2.3 g/100 ml; total protein 5.3 g/100 ml; Na^+ 146 mEq/liter; K^+ 3.2 mEq/liter; Osm 293 mOsm/kg) with a small amount of Lissamine green and one μ Ci/ml of ^{14}C-carboxyl inulin (New England Nuclear Corp., Boston, Mass.) was used to perfuse the capillaries in 16 rats. The purpose of the Lissamine green in the perfusion fluid was to provide a visual evaluation of the technical adequacy of the perfusion and the purpose of the ^{14}C-inulin was to detect any contamination of the free flow tubular fluid with the perfusion fluid due to inadvertent direct puncture of the tubular lumen by the perfusion pipet. In two additional rats, a hyperoncotic perfusion fluid was used to perfuse the peritubular capillaries. This fluid was prepared by ultra filtration of pooled rat plasma by means of an Amicon ultrafiltration cell (Amicon Corp., Lexington, Mass.) Model 10-PA, with PM-30 diaflo membrane. The original volume of plasma was reduced by 50%. The protein concentration of the final solution was 11.6 g/100 ml. Lissamine green and ^{14}C-inulin were added, as in the other perfusion solution.

The protocol was as follows: a micropipet and an attached 18 inch length of thick-walled PE 20 polyethylene tubing were filled with the perfusion plasma and mounted on a micromanipulator. The free end of the polyethylene tubing was connected to either a tank of 5% nitrogen equipped with a reduction valve (12 experiments) or to a 250 μliter Hamilton syringe mounted on a Sage pump (Model 255–2) (six experiments). The perfusion pipet was inserted into the central arteriole of a vascular "star" and perfusion of the capillary network was started by means of either an applied hydrostatic pressure from the gas tank or volume displacement from the Sage pump. About 15 to 30 sec later, one of the end proximal convolutions immediately adjacent to the vascular "star" was punctured with a second pipet filled with castor oil colored with Sudan black. A long column of the oil was injected from this pipet and allowed to flow distally. The front of this column usually descended beneath the surface of the kidney and did not reappear in any other surface convolution. A timed collection of tubular fluid was then made, usually lasting two to four minutes, the pipet withdrawn into mineral oil bathing the surface of the kidney, and the tip of the pipet sealed by drawing in some of the mineral oil. A second collecting pipet was subsequently inserted into the same convolution (usually via the hole left by the first collecting pipet) and a second timed tubular fluid collection was made after the flow rate out of the perfusion pipet had been increased or decreased.

Table 1. Comparison of ^{14}C-and ^{3}H-Inulin clearances in a single rat[a]

Clearance period	^{14}C-Inulin *ml/min/kg*	^{3}H-Inulin *ml/min/kg*
1	11.38	11.46
2	11.97	11.76
3	11.38	11.28
4	13.41	13.44
5	14.17	13.93

[a] Urine collections were made from the exposed urinary bladder.

Usually, the oil column injected with the first collecting pipet remained in place, probably due to leakage of tubular fluid from a hole in the tubular epithelium left by the first pipet. Occasionally, the oil column would disappear beneath the surface after the first pipet was removed, so that a new oil column had to be injected with the second collecting pipet. In general, the capillary perfusion rate was changed about three to four min before the second collecting pipet was inserted into the tubule, in order to allow the perfusion rate to equilibrate at the new level. Preliminary tests carried out *in vitro* showed that constant rates were achieved approximately three to four min after the gas pressure or perfusion pump setting was changed. The second tubular fluid collection also lasted two to four min. The area of the kidney surface reached by the perfusion fluid was not measured, but at the lower perfusion rates the colored plasma usually surrounded 8 to 12 convolutions in the immediate vicinity of the central arteriole. Care was taken to select a terminal convolution for micropuncture which was definitely perfused by the colored plasma. When the perfusion rate was raised, the area of perfusion was observed to increase. If this did not occur, we assumed that the perfusion pipet had become partially plugged, did not proceed with a second tubular collection, and discarded the first collection. Arterialized blood samples of about 100 µliter each were collected in heparinized capillary tubes from the cut tail at about 30 min intervals throughout the experiment.

The total volume of each tubular fluid sample was determined by transferring the sample into toluene in the tip of a constant-bore capillary tube and measuring the length of the column with an eyepiece micrometer [16]. Aliquots of plasma, urine and the perfusion fluid were transferred to the same capillary tube for volume measurement. All samples were washed out of the capillary tube with five drops of water into a liquid scintillation counting vial containing ten ml of a scintillation cocktail made up of 88 % toluene, 9 % Bio-Solv Solubilizer (Beckman Instruments, Inc.) and 3 % Liquifluor (New England Nuclear Corp.). The samples were analyzed for ^{14}C and ^{3}H in a Nuclear-Chicago liquid scintillation counter, Unilux IIA. The channels were set so that ^{3}H was excluded from the ^{14}C channel. Both ^{14}C and ^{3}H *dpm* were calculated by the channels ratio method, utilizing ^{133}Ba as an external standard. Because of the very low plasma levels of ^{14}C, the plasma and tubular fluid samples were counted for a minimum of 200 min.

Single nephron GFR (SNGFR) was calculated from:

$$\text{SNGFR} = \frac{\text{total } ^{3}H \text{ in the tubular sample/min collection}}{\text{Plasma } [^{3}H]} \quad (1)$$

assuming a plasma water content of 94 %. The amount of ^{14}C expected in the tubular fluid from glomerular filtration was calculated from:

$$\text{Expected } ^{14}C = \text{SNGFR} \times \text{Plasma } [^{14}C]. \quad (2)$$

In approximately 1/3 of the plasma samples, ^{14}C-activity could not be detected above background, and it was assumed therefore that any ^{14}C found in the tubular fluid represented contamination by perfusion fluid. In the majority of instances, however, plasma ^{14}C reached high enough levels to be measured, and the amount expected in the tubular fluid from filtration was calculated according to Equation (2). Any tubular fluid sample which contained more ^{14}C than could be attributed to glomerular filtration was discarded, since the excess might have been due to inadvertent puncture of the tubular lumen by the capillary perfusion pipet. Although it is possible that in some cases, excess ^{14}C appeared in the tubular collections due to retrograde flow of perfusion fluid from the efferent arteriole to the glomerulus, and filtration across the glomerulus, this method of analysis does not distinguish between this route and direct contamination of the lumen distal to the glomerulus.

Per cent reabsorption of filtered water was calculated from:

$$\% \text{ reabsorption} = \frac{\text{SNGFR} - \text{Collection rate}}{\text{SNRFR}} \times 100 \quad (3)$$

and absolute fluid reabsorption from:

$$\text{nl/min} = \text{SNGFR} - \text{Collection rate}. \quad (4)$$

All calculations were carried out with appropriate computer programs on an Olivetti Programma 101. Statistical analysis by unpaired and paired "t" tests was carried out according to the methods described by Steel and Torrie [17].

Calibration experiments were carried out to determine the flow rate of plasma out of the perfusion micropipet with both the gas pressure system and the Sage pump. For testing the gas pressure system, a series of micropipets were prepared with a range in tip diameter (O. D.) from 13 to 15.5 µ. Flow rate of normal rat plasma was determined by delivering the plasma from the micropipet into a counting vial and measuring the ratio of the ^{14}C counts in the vial to that of a measured volume of the plasma. Flow rates were determined over a range of applied pressures comparable to those used in the rat experiments. The Sage pump was also calibrated with ^{14}C-inulin containing normal plasma and hyperoncotic plasma.

Results

In Fig. 1 are shown the results of *in vitro* flow measurements of rat plasma from micropipets of different tip diameters at different applied hydrostatic pressures. As can be seen, flow rate was markedly influenced by the tip diameter. At an applied pressure of four PSI, for example, flow rate ranged from 260 to 800 nl/min with tip diameters ranging from 13 to 15.5 O.D. At a PSI of eight, the range of flow with the same pipets was from 400 to 1 280 nl/min. It is also apparent that the slope of the pressure-flow

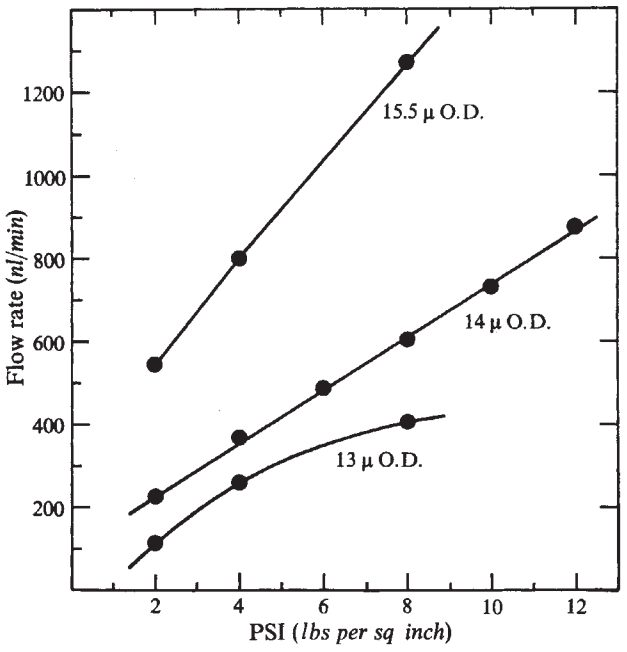

Fig. 1. *Flow rates of plasma from micropipets of different tip diameters at various applied pressure (PSI), measured in vitro*

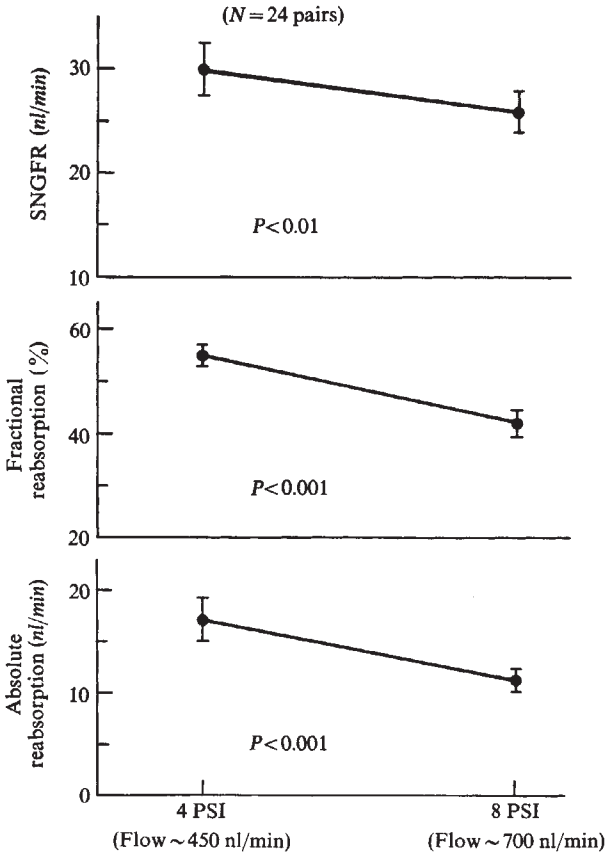

Fig. 2. *Recollection micropuncture data obtained during perfusion of the peritubular capillaries: Gas pressure system.* The perfusate was rat plasma (protein 5.3 g/100 ml). A gas pressure system was used to control the rate of perfusion. Data expressed as mean ± SEM

relationship varied with the tip diameter, such that for a given increase in applied pressure, a greater rise in flow rate occurred with larger pipets. These findings are predictable, and indicate that in the group of capillary perfusion experiments using the gas pressure system, the range of perfusion rates was probably large. Although no systematic attempt was made to produce perfusion pipets of a uniform diameter in the rat experiments, measurement of a random sample of these pipets yielded an average value of 14.4 μ with a range from 13.5 to 15 μ O.D. This suggests that flow rates probably ranged between about 300 and 600 nl/min at a PSI of four (mean: 450 nl/min) and between 500 and 900 nl/min at eight PSI (mean: 700 nl/min). The lower value of about 450 nl/min is considerably higher than the estimated normal blood flow through a single postglomerular arteriole (about 230 to 250 nl/min). However, such a rate of perfusion was necessary to clear the surrounding capillaries of blood and to assure that at least several convolutions of the nephron would be in contact with the perfusate.

In Table 2 and Fig. 2 are shown the results of experiments in 12 rats in which peritubular capillaries were perfused with pooled rat plasma, using the gas pressure system. Single nephron glomerular filtration rate (SNGFR), fractional reabsorption of water, and absolute rate of fluid absorption are compared in recollections from the same convolution during two different perfusion pressures. The lower perfusion pressure was usually four PSI (range: 3 to 5) and the higher pressure was usually eight PSI (range: 7 to 9). Those collections marked with + in Table 2 were carried out in a reverse sequence, i.e., at the higher perfusion pressure first, then at the lower pressure. Paired "t" test analysis of SNGFR revealed that filtration rate fell significantly ($P < 0.01$) when the capillaries were perfused at the higher pressure. Although this did not occur in seven of the 24 tubules, a trend seemed clear. It seems unlikely that the change in SNGFR was due to an artifact of the recollection technique, since the artifact that has been described to occur with this technique is a rise in SNGFR during the second collection [18, 19].

Per cent reabsorption of filtered water fell in every instance (only slightly in one case) when the capillaries were perfused at the higher pressure. The mean value at the lower pressure was 56.4% ± SEM 2.0 and at the higher pressure was 38.1% ± SEM 3.9 ($P < 0.001$). Similarly, absolute absorption rates fell from 16.8 nl/min ± SEM 1.5 to 9.28 nl/min ± SEM 1.2 ($P < 0.001$). That the fall was not related to the recollection technique *per se* is suggested by the four instances in which the capillaries were perfused at the higher pressure first, and then at the lower pressure. In each of these four tubules, fractional reabsorption rose during the second tubular fluid collection when the capillary perfusion pressure was lower.

Table 2. Free flow recollections from end-proximal tubules during microperfusion of peritubular capillaries. Gas pressure system

Rat No.	Tubule No.	SNGFR *nl/min*		Diff. *nl/min*	Fractional reabsorption %		Absolute reabsorption *nl/min*	
		L[a]	H[a]		L	H	L	H
1	1	37.07	26.31	− 10.76	57	48	21.25	12.91
2	2	52.75	20.86	− 31.89	52	4	30.63	0.92
3	3	9.71	10.05	+ 0.34	63	56	6.13	5.60
	4	15.51	20.96	+ 5.45	87	82	13.56	17.63
	5	22.11	16.47	− 5.64	63	31	14.34	5.53
	6	19.63	23.81	+ 4.18	46	20	8.90	4.86
4	7	41.15	41.58	+ 0.43	51	44	21.31	18.54
	8	43.54	41.82	− 1.72	47	29	20.51	12.08
5	9	33.10	24.72	− 8.38	53	14	18.58	3.82
	10	35.33	32.55	− 2.78	50	40	18.02	13.11
6	11[b]	21.91	20.80	− 1.11	48	37	10.91	7.90
	12[b]	29.43	33.57	+ 4.14	57	48	18.49	18.22
7	13	22.26	20.38	− 1.88	51	33	11.82	7.03
	14	16.31	11.88	− 4.43	59	53	9.77	6.35
8	15	31.16	28.12	− 3.04	69	60	21.62	17.10
9	16	19.43	15.30	− 4.13	45	28	8.80	4.23
	17	29.81	6.00	− 23.81	57	8	17.92	0.51
10	18	14.41	11.98	− 2.43	55	21	8.68	2.54
	19	45.62	26.96	− 18.66	76	78	38.19	21.04
11	20[b]	21.43	18.79	− 2.64	52	37	11.34	6.97
	21	35.19	19.11	− 16.08	53	41	18.52	8.14
	22	38.56	31.79	− 6.77	59	37	22.72	11.82
12	23[b]	26.82	23.08	− 3.74	52	37	14.14	8.48
	24	33.12	25.06	− 8.06	52	29	17.22	7.39
Mean		28.97	23.00	− 5.98	56	38	16.81	9.28
SEM				1.81	1.99	3.90	1.50	1.20
P				< 0.01	< 0.001		< 0.001	

[a] Capillary perfusion rate was changed by applied gas pressure in these experiments. L refers to the lower pressure (3 to 5 PSI) and H the higher pressure (7 to 9 PSI).

[b] In these collections, the capillaries were perfused at the higher pressure first, then the lower pressure. The *P* value for SNGFR was calculated from "student's t" test for paired samples and represents the probability of the mean change being different from zero.

Table 3. Free flow recollections from end-proximal tubules during microperfusion of peritubular capillaries. Perfusion pump system

Rat No.	Tubule No.	SNGFR *nl/min*		Diff. *nl/min*	Fractional reabsorption %		Absolute reabsorption *nl/min*	
		400 nl/min	800 nl/min		400 nl/min	800 nl/min	400 nl/min	800 nl/min
13	1[a]	26.17	17.64	− 8.53	55	24	14.39	4.39
	2[a]	18.71	20.92	+ 2.21	48	47	9.06	9.92
	3	50.59	29.84	− 20.75	69	41	35.22	12.50
14	4[a]	33.51	29.72	− 3.79	59	40	19.85	11.61
	5	26.39	23.66	− 2.73	62	46	16.41	10.85
	6[a]	30.93	34.84	+ 3.91	59	48	18.18	16.62
	7[a]	27.10	29.20	+ 2.10	48	38	13.12	11.11
	8	36.40	29.93	− 6.47	53	48	19.61	14.50
15	9	38.35	33.11	− 5.24	63	56	24.17	18.39
	10	19.18	15.18	− 4.00	47	30	9.02	4.97
16	11	25.23	22.46	− 2.77	47	40	11.98	9.03
	12	27.02	22.73	− 4.29	54	51	14.63	11.69
Mean		29.97	25.77	− 4.20	55	42	17.14	11.30
SEM		2.55	1.79	1.85	2.08	2.58	2.09	1.19
P				< 0.05	< 0.001		0.05 > *P* > 0.02	

[a] In these collections, the capillaries were perfused at the higher rate first, then the lower rate.

In Table 3 and Fig. 3 are shown the data obtained in four rats in which the capillaries were perfused with pooled rat plasma at two known rates by means of the Sage pump. As can be seen, SNGFR showed variable changes but it was significantly lower at the higher perfusion rate when analyzed by paired "t" test. Fractional and absolute water reabsorption were also significantly lower. These observations are in accord with those made in the larger group of animals in which the gas pressure system was used. As shown in Table 3, when the order of perfusion rate was reversed with the Sage pump, fractional reabsorption rose in four out of five tubules during the second collection when capillary perfusion rate was slower.

In Table 4, the data from the 9 tubules (Tables 2 and 3) in which the capillaries were perfused at the faster rate first and then at the slower rate have been analyzed by paired "t" test [17]. As can be seen, both fractional and absolute reabsorption increased significantly during the second tubular fluid collection, in sharp contrast with the rest of the observations in which the opposite sequence of capillary perfusion was used. This suggests that the results were not due to a non-specific effect of peritubular capillary perfusion, such as progressive anoxia or depletion of a substrate essential for sodium transport. The observations also provide evidence that the recollection method was not in itself responsible for the changes in fractional and absolute reabsorption. SNGFR did not change significantly in this small series of observations.

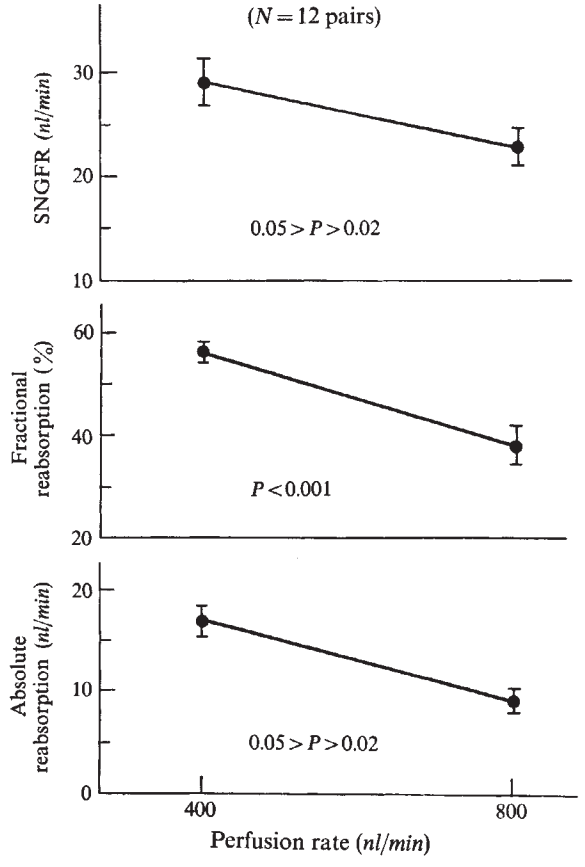

Fig. 3. *Recollection micropuncture data obtained during perfusion of the peritubular capillaries: Perfusion pump system.* The perfusate was rat plasma (protein 5.3 g/100 ml). A Sage pump was used to control the rate of perfusion. Data expressed as mean ± SEM

Table 4. Recollection data in which capillaries were perfused first at a high rate, followed by a lower rate[a]

	High rate	Low rate	Diff.	Paired "t" test *P*
SNGFR, *nl/min*	25.4 ± 2.1	26.2 ± 1.6	+ 0.8	NS
% Reabsorption	39.6 ± 2.5	53.1 ± 1.5	+13.5	<0.01
Absolute reabsorption, *nl/min*	10.6 ± 1.5	14.4 ± 1.3	+ 3.8	<0.02

[a] Perfusion fluid was rat plasma with protein concentration 5.3 g/100 ml. Data are from nine tubules in Tables 2 and 3 and are presented as mean ± SEM.

In Fig. 4 are shown the recollection data obtained in ten tubules in which the peritubular capillaries were perfused with hyperoncotic rat plasma by means of the Sage pump. The lines connect points from individual nephrons. It is clear that fractional and absolute reabsorption fell in nine out of the ten tubules when the perfusion rate was increased from 400 to 800 nl/min, although the changes were somewhat smaller than were observed when plasma with 5.3 g/100 ml protein was used. The data are summarized in Table 5 where both unpaired and paired "t" test analysis has been used. The changes in fractional and absolute reabsorption were statistically significant by both methods of analysis, whereas SNGFR changes were not.

Discussion

The results of the present experiments indicate that changes in peritubular capillary flow rate lead to changes in rate of sodium and water reabsorption by the proximal convoluted tubule. This conclusion is based upon observations made with a capillary microperfusion technique and the recollection method of tubular sampling, both of

Table 5. Free-flow recollections from end-proximal tubules during microperfusion of peritubular capillaries with hyperoncotic plasma[a]

	Perfusion rate		Unpaired "t" test	Paired "t" test
	400 *nl/min*	800 *nl/min*	*P*	*P*
SNGFR, *nl/min*	24.1 ± 1.3	22.7 ± 0.9	NS	NS
% Reabsorption	56.4 ± 1.7	46.4 ± 2.3	<0.01	<0.01
Absolute reabsorption, *nl/min*	13.8 ± 1.1	10.7 ± 0.9	<0.05	<0.01

[a] Perfusion fluid was rat plasma with a protein concentration of 11.6 gm%, prepared by ultrafiltration. Data expressed as mean ± SEM.

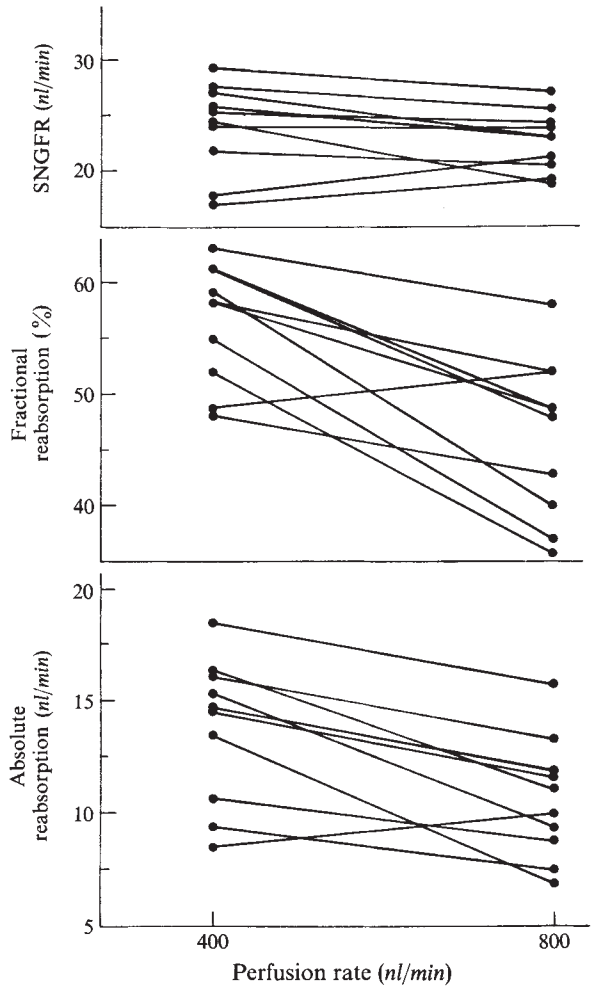

Fig. 4. *Recollection micropuncture data obtained during perfusion of the peritubular capillaries.* The perfusate was hyperoncotic rat plasma with a protein concentration of 11.6 g/100 ml. A Sage pump was used to control the perfusion rate. Each line connects points from a single nephron.

which have several potential sources of error. First, if the perfusion pipet accidentally punctures the tubular epithelium, the perfusion fluid would contaminate the tubular fluid. This would dilute the concentration of filtered inulin, increase the volume of collected fluid, and obviously lead to erroneous values for fractional and absolute reabsorption. In order to detect any such accidental punctures, we added ^{14}C-inulin to the perfusion fluid and discarded any tubular collections in which there was more ^{14}C-inulin than could be accounted for by the level of ^{14}C in the peripheral blood and SNGFR (Equation (2)). Fifteen samples out of a total of 107 collections had to be discarded because of this technical problem, indicating the need for such monitoring. A second problem with the capillary perfusion method relates to the direction of flow of the perfusion fluid. It has been shown that the blood in the central arteriole of vascular "stars" comes from the glomerulus beneath the surface, and normally flows outward into the primary branches of the star [2, 15]. Steinhausen, Eisenbach, and Galaske [15] noted that for most of the surface proximal convolutions, blood flow is counter to the direction of tubular fluid flow and they have postulated that this counter-current flow may be important for glomerulotubular balance. Whether or not this proves to be the case, artificial perfusion of the postglomerular capillaries is probably more physiologic if the perfusion fluid flows in the same direction as natural blood flow. For this reason, we perfused the central arterioles. A third problem is that the recollection method of micropuncture may cause certain artifactual changes during the second tubular fluid collection [18, 19]. However, as it was found that fluid reabsorption either decreased or increased during the second tubular collection, depending upon the rate of capillary perfusion, the data cannot be attributed to a recollection artifact. The data in nine tubules in which sodium reabsorption increased during the second tubular collection (Table 4) as capillary perfusion rate was lowered also provide evidence against a non-specific effect of artificial capillary perfusion, such as diminished oxygen supply to the tubular epithelium. A progressive decline in sodium reabsorption might be expected if this were the case. We assume, therefore, that the changes in fractional and absolute reabsorption observed in these experiments were not due to technical artifacts but were the result of the changes in capillary perfusion rate.

Since SNGFR changed in many of the recollection pairs, we must consider the possibility that changes in the filtered volume were responsible for the changes in sodium reabsorption. However, this does not seem to be the case. First, in the data shown in Tables 4 and 5, SNGFR did not change significantly whereas absolute and fractional sodium reabsorption did. Second, in those experiments in which SNGFR did change appreciably (Tables 2 and 3), the direction of change was opposite to that which might be expected to produce the observed change in fractional reabsorption. For example, when GFR is reduced by constricting the aorta or renal artery, fractional reabsorption usually rises slightly [1, 4, 5]. In the present study, when GFR did fall, this was usually accompanied by a fall in fractional sodium reabsorption. Thus, the changes in sodium reabsorption did not seem to be related to changes in GFR. The observations suggest rather that under the present experimental conditions, the volume of fluid entering the tubular lumen at the glomerulus was not the critical factor which caused the changes in fractional reabsorption.

Although changes of GFR did not seem to be responsible for the changes in sodium reabsorption, it is possible that the converse was true. We suspect that SNGFR tended to fall when sodium and water reabsorption was inhibited markedly. In the capillary perfusion experiments of Spitzer and Windhager [12] and Brenner and Troy [20], analysis of their data by paired "t" test shows that SNGFR fell significantly ($P < 0.01$) when sodium reabsorption was inhibited due to perfusion with colloid-free Ringer's solution. When the capillaries were perfused with dextran or

protein-containing solutions, sodium reabsorption was not inhibited and SNGFR did not change significantly from control values. These calculations, together with the observations shown in Tables 2 and 3, suggest that inhibition of proximal sodium and water reabsorption during capillary perfusion may be responsible for a fall in SNGFR. The mechanism might be a rise in intratubular pressure and a subsequent reduction in effective filtration pressure.

Proximal sodium reabsorption has been found to respond to changes in the colloid oncotic pressure of the peritubular fluid [12, 20]. We must consider the possibility, therefore, that in the experiments in which pooled rat plasma with a protein concentration of 5.3 g/100 ml was used to perfuse the peritubular capillaries (Tables 2 and 3), the inhibition of sodium and water reabsorption was due to dilution of the normally higher protein concentration of peritubular capillary blood. Because water but not protein is filtered at the glomerulus, the protein concentration in postglomerular blood is normally about 9 to 10 g/100 ml [20]. Thus, perfusion of the postglomerular capillaries with a solution of lower protein concentration might inhibit sodium reabsorption due to a colloid oncotic effect [12]. It seems unlikely, however, that dilution of postglomerular proteins can entirely account for the observations. In the study by Spitzer and Windhager [12], it was found that absolute rates of proximal sodium reabsorption fell by approximately 50% in both free-flow tubular collections and split oil droplet measurements when normal peritubular blood was replaced by protein-free Ringer's solution. These authors estimated that the normal reabsorptive rate would change by about 16% for each 10 mm Hg change in protein oncotic pressure [12]. If we assume that the pooled rat plasma used in the experiments shown in Tables 2 and 3 diluted peritubular proteins from 9 to 10 g/100 ml to 5.3 g/100 ml, oncotic pressure would fall by about 20 mm Hg [21], and this might be expected to produce a 32% fall in absolute sodium reabsorption [12]. The decrease in absolute reabsorption between the low and high perfusion rates averaged 45% and 34%, respectively, for the two perfusion techniques (Tables 2 and 3). However, in order to attribute this to a colloid oncotic change, one would have to assume that none of the proximal tubular convolutions were perfused during the lower perfusion rate and that most or all of the convolutions were reached by the perfusion fluid at the higher rate. Since visual inspection indicated that many convolutions were surrounded by the perfused plasma at the lower perfusion rates, it seems unlikely that the marked inhibition of reabsorption at higher perfusion rates was due entirely to a dilution of normal peritubular capillary proteins.

In order to examine this possibility more rigorously, we carried out additional experiments in which hyperoncotic rat plasma was used to perfuse the peritubular capillaries (protein concentration: 11.6 g/100 ml). The data, shown in Fig. 4 and Table 5, clearly demonstrate that absolute and fractional reabsorption were reduced when the perfusion rate was raised from 400 to 800 nl/min. Since the protein concentration of the capillary blood would not have been diluted with this hyperoncotic solution, these results cannot be attributed to a colloid oncotic presssure effect. We assume, therefore, that the fall in reabsorption was related to the increase in perfusion rate *per se* or to some effect produced by the increase in perfusion rate.

Although the precise mechanism responsible for the inhibition of sodium reabsorption is not certain, several different possibilities can be considered. First, flow rate in the capillaries may in itself effect sodium reabsorption. Buentig and Earley [6] postulated that proximal reabsorptive rate might be related to the total peritubular oncotic force, which is a function of both the concentration of proteins in the peritubular plasma and the rate of flow through the capillaries. They suggested that during constriction of the renal artery, reduced proximal sodium reabsorption might be due to the slower flow rates in the peritubular capillaries. This explanation can not account for the present observations, however, since increased perfusion rates were associated with a decrease in sodium reabsorption. A second possibility is that a chemical inhibitor of sodium reabsorption is normally present in rat plasma, and that larger amounts of this substance were delivered to the tubular epithelium when the perfusion rate was increased. Since the pooled rat plasma used in the perfusions was obtained from non-diuretic rats, there is no reason to postulate the presence of a "natriuretic" hormone, although the possibility cannot be excluded that even hydropenic plasma contains small amounts of such a hormone. If this were the case, the molecular weight of the hormone should be above 30,000, since the phenomenon was observed with plasma that had been exposed to a PM-30 Diaflo membrane.

A third possibility is that the increase in perfusion rate caused a rise in peritubular hydrostatic pressure, and that this pressure increase was in itself responsible for the fall in sodium reabsorption. A number of previous studies [8, 13, 22–27] have shown that acute changes in the perfusion pressure of the kidney can alter the rate of sodium reabsorption by the tubules, without necessarily inducing any overall change in GFR or renal blood flow. Recent improved techniques for measuring hydrostatic pressures in the kidney have revealed that under normal hydropenic conditions, pressure in the small peritubular capillaries is significantly lower than in the lumen of the proximal tubule [28]. Interstitial pressure is assumed to be slightly lower than the capillary pressure [28]. Thus, in contrast to earlier data, the recent measurements of hydrostatic pressure indicate that gradients do exist between the tubular lumen and capillary, with the interstitium perhaps having the lowest pressure of all. It seems possible that when the perfusion rate was increased, the uptake of interstitial fluid by the capillaries was reduced due to a rise in hydrostatic pressure in the capillaries. The resulting expansion of the interstitium would be expected to cause an abrupt rise of the pressure within this normally low pressure compartment. This in

turn might conceivably have led to hydrodynamic changes in the lateral intercellular channels which Diamond and Bossert have proposed to be important for the regulation of epithelial transport rates [29]. Although the present data do not distinguish among the mechanisms discussed above, they seem to be most compatible with the view that hydrostatic pressure changes initiated the observed changes in absolute and fractional sodium reabsorption by the proximal convoluted tubule.

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